[C1-091]

Comparative transcriptomics of the response of *Mycobacterium bovis* BCG to sodium hypochlorite, hydrogen peroxide and peracetic acid.

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Introduction

-Tuberculosis is a leading cause of death worldwide and infects thousands of Americans annually.

-*M. bovis* is part of the *M. tuberculosis* complex and is implicated in tuberculosis in humans and several animal species.

-The *M. bovis* bacillus Calmette-Guerin strain (*M. bovis* BCG) that is widely used as a human vaccine against tuberculosis was derived from *M. bovis*.

-Sodium hypochlorite, hydrogen peroxide and peracetic acid are EPA-approved oxidative disinfectants for the eradication of pathogens including *M. tuberculosis* in the hospital and domestic environments.

- A comparative analysis of the toxicogenomic responses of any mycobacterial species to different antimicrobials has not been previously carried out.

Objective of Study

To compare the global transcriptomic responses of *M. bovis* BCG to sodium hypochlorite, hydrogen peroxide and peracetic acid after 10 minutes of treatment.

Materials and Methods

A: Growth Inhibition of *M. bovis* BCG with Sodium hypochlorite, hydrogen peroxide and Peracetic acid

-ATP measurements used to monitor changes in growth after sodium hypochlorite, hydrogen peroxide and peracetic acid exposure.

-The Bac-titer Glo™ microbial cell viability assay and the Glomax™ luminometer were used for ATP measurements.

-Three separate microarray experiments were performed in the absence (controls) and in the presence of 2.5mM Sodium hypochlorite (Jang et al. 2009a), 0.5mM hydrogen peroxide (Jang et al. 2009b) and 0.1mM peracetic acid for 10 minutes.

-Microarray results were validated using quantitative real time PCR.

Statistical Analysis of Microarray Data

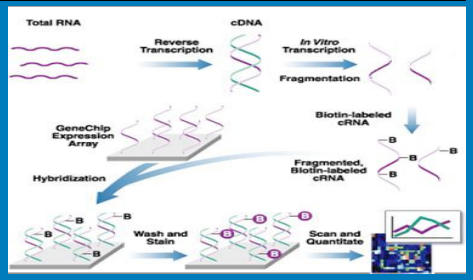
-p-value for 1-Way ANOVA ≤ 0.05

-Fold change in transcript level ≥ 2.0

-Presence or marginal calls ≥ 50% replicates in both the experimental and control sets

-The array data for treatments with sodium hypochlorite, hydrogen peroxide and peracetic acid have been deposited in the National Center for Biotechnology information (NCBI) gene Expression Omnibus and is accessible through series numbers GSE 13423, 14272 and 15023.

DNA Microarray Procedure Courtesy of Affymetrix



Results and Discussion

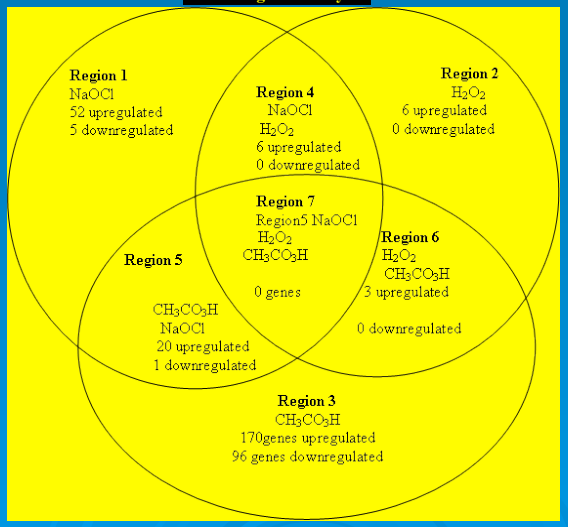
-Of the 5412 genes represented in the *M. bovis* BCG custom array, 4,860 genes passed the present/marginal call to form a master list.

-Based on the one-way ANOVA, 2090, 2069 and 1973 genes in the sodium hypochlorite, hydrogen peroxide and peracetic acid sample sets respectively were statistically significant.

-When fold change analysis was carried out:

- 84 genes in the sodium hypochorite treated samples showed a 2 fold or more up or downregulation in expression compared to the control samples.
- Fifteen genes in the hydrogen peroxide treated samples showed a 2 fold or more up or downregulation in expression compared to the control samples
- Within the peracetic acid treated samples, 290 genes were 2 fold or more up or downregulated compared to the controls.

Venn Diagram Analysis



Region 4:Genes up and downregulated in common between sodium hypochlorite and hydrogen peroxide

-Five of the upregulated genes in this region were hypothetical proteins and the sixth gene was an intergenic region with no known function.

Region 5: Genes up and downregulated in common between sodium hypochlorite and peracetic acid

-The *ctp*F gene which encodes a putative metal cation transporter P-type atpase A was the only gene with a known function in this region.

-The *ctp*F, gene has been shown to be upregulated in *M. tuberculosis* in response to exposure to reactive nitrogen intermediates

-Further, the *ctp*F gene was upregulated in *M. tuberculosis* in response to growth in a hypoxic environment.

Region 6: Genes up and downregulated in common between hydrogen peroxide and peracetic acid

-The *kat*G gene which encodes a catalase-peroxidase-peroxynitritase T enzyme was upregulated in this region.

-The *kat*G gene is a hallmark anti oxidative stress enzyme.

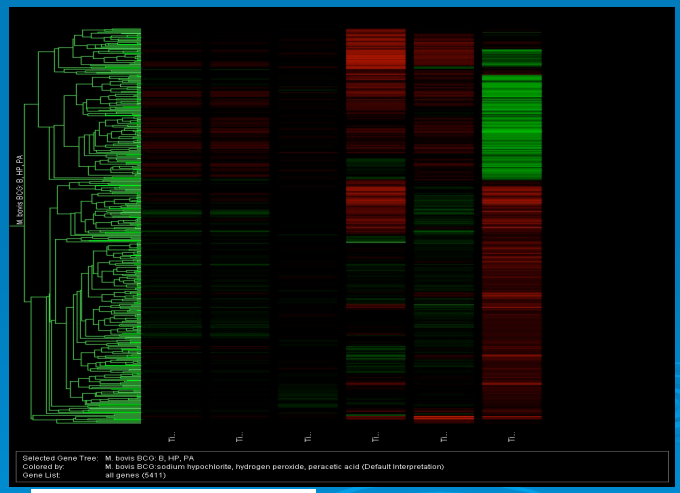
-This region also contained the *mbt*D and *mbt*I genes which encode polyketide synthases involved in the biosynthesis of mycobactins.

-Mycobactins are salicylic acid-derived siderophores, important in mycobacterial iron acquisition/virulence

Region 7: Genes up and downregulated in common among all three disinfectants

No genes were upregulated in common between all three disinfectants.

Heat Map Analysis



Lane 1: Control samples: sodium hypochlorite
Lane 2: Control samples: hydrogen peroxide
Lane 3: Control samples: peracetic acid
Lane 4: 10 minute samples: sodium hypochlorite
Lane 5: 10 minute samples: hydrogen peroxide
Lane 6: 10 minute samples: peracetic acid

Red: Upregulated genes
Green: Downregulated genes

Conclusions

-Sodium hypochlorite and peracetic acid treatments led to more changes in gene expression (up and downregulation of genes) compared to hydrogen peroxide treatment.

-Although the three disinfectants were oxidative, there were no genes upregulated in common among them.

-The upregulation of *kat*G, *mbt*D and *mbt*I supports the connection between iron regulation and oxidative stress response in *M. bovis* BCG exposed to both hydrogen peroxide and peracetic acid.